Attachment A

Emulsified Intravenous Versus Evaporated Inhaled Isoflurane for Heart Protection: Old Wine in a New Bottle or True Innovation?

Eliana Lucchinetti, PhD*†

Marcus C. Schaub, MD, PhD‡

Michael Zaugg, MD, DEAA*†

In his novel entitled "Clothes make the man," Zurich novelist Gottfried Keller moralizes upon the enormous impact of fine clothing on social status and reputation in the Swiss bourgeoisie of the second half of the nineteenth century (1866). Likewise, encapsulation or formulation of a medication (galenics) and the route of administration are known to profoundly affect pharmacokinetic and/or dynamic properties of a drug, to modify the ratio between therapeutic activities versus toxicity (therapeutic index), and are even capable of evoking novel biological actions.[1,2] In this issue of Anesthesia & Analgesia, Rao et al.[3] report on the protective properties elicited by evaporated and inhaled versus emulsified IV isoflurane in an in vivo rabbit model of regional ischemia–reperfusion of the heart. Both isoflurane preparations were administered on top of a pentobarbital background anesthesia using a classical preconditioning protocol with washout before index ischemia, and equally reduced infarct size by approximately 50%.

Apart from the peculiar but tantalizing idea of injecting volatile gases, anesthesiologists will, of course, immediately recognize the great potential of this novel concept. First, IV administration eliminates the need for specific ventilatory circuits and renders anesthetic induction independent of pulmonary function, in particular functional residual capacity. Second, preliminary data from large animal experiments provide evidence that anesthetic induction and recovery are more rapid for emulsified IV isoflurane compared with propofol,[4] and further demonstrate a remarkable hemodynamic stability[5]; two advantages, which are supplemented by lower amounts of drug metabolism (isoflurane approximately 0.2% vs propofol approximately 100%). Also, since IV, as opposed to inhaled administration, requires significantly less isoflurane (approximately 80% less for induction and approximately 20% less for maintenance) to obtain comparable anesthetic and organ-protective effects,[6] reduced environmental pollution and tissue toxicity originating from oxidative metabolites such as acetylated proteins[7] should be expected. Third, emulsified isoflurane with its preconditioning effects could be added to organ-preserving solutions. Finally, since volatile anesthetics elicit protection of the endothelium, a key component of all body tissues, at even lower subanesthetic doses,[8] IV administration, would clearly facilitate the use of halogenated ethers for organ protection in other clinical fields, and thus promote their administration during diagnostic and interventional procedures in cardiology or endovascular procedures of high-risk patients.

The first report on the hazards of methoxyflurane emulsions backdates to 1968.[9] Since then, pharmacological research in different small and large animal species clarified most of the pharmacokinetic issues and further showed a similar safety profile of emulsified versus inhaled isoflurane if the formulation was properly prepared.[10,11] Interestingly, isoflurane as 8% Intralipid® emulsion was also successfully used for IV regional anesthesia (Bier's block) in rats, providing comparable anesthetic effects as 1%

From the *Institute of Anesthesiology, E-HOF, University Hospital Zurich, †Zurich Center of Integrative Human Physiology (ZIHP), and ‡Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland.

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Address correspondence and reprint requests to Privatdozent Dr. Michael Zaugg, Institute of Anesthesiology, E-HOF, University Hospital Zurich, and Zurich Center of Integrative Human Physiology (ZIHP), University of Zurich, Rämistrasse 100, CH-8091 Zurich, Switzerland. Address e-mail to michael.zaugg@usz.ch.

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lidocaine,[12] and for intrathecal administration in dogs,[13] showing dose-dependent increases in motor and sensory blocks without clinical toxicity. Finally, 8% emulsified isoflurane supplemented to cardioplegia (4°C) enhanced cardiac protection in isolated rat hearts and reduced infarct size by an additional 23%.[14] One of the most important studies on IV emulsified halogenated ethers and heart protection was conducted by Chiari et al.[15] In this study, pentobarbital-anesthetized rabbits were treated with a lipid vehicle alone consisting of soy bean oil and egg lecithin or with emulsified ethers (isoflurane 6.9%, enflurane 7.1%, and sevoflurane 7.5%) for 30 min using a classic preconditioning protocol with 30 min washout time. The hearts were subjected to 30 min of coronary artery occlusion and 3 h of reperfusion. While the lipid vehicle did not reduce infarct size compared with saline control, emulsified ethers reduced infarct size by approximately 50%. Two findings of this study are remarkable. First, similar reduction in infarct size was achieved by all halogenated ethers in the "early" preconditioning protocols. Second, emulsified sevoflurane also elicited a "second window of protection" and reduced infarct size by approximately 50% 24 h later. In this protocol, sevoflurane at end-tidal concentration as low as 0.34 vol %, corresponding to 0.63 vol % blood concentration or approximately 0.17 MAC, was effective, notably without sedation or respiratory depression during IV administration.

These findings raise the intriguing possibility that organ-protective actions elicited by halogenated ethers may be largely separated from anesthetic effects if administered as emulsions. Separation of hypnotic from analgesic effects was reported for emulsified ethers (isoflurane, enflurane, and sevoflurane) in a mouse model of nociception.[16] After intraperitoneal injection of emulsified ethers, tail-withdrawal tests showed increased latency, which was abolished by the intrathecally administered glycine receptor blocker strychnine, but there was no effect on sleeping time. Sevoflurane administration by inhalation is accompanied by considerable sedation at even low concentrations (bispectral index approximately 70 with 1 vol % ≈0.5 MAC),[8,17] for which, however, effective endothelial protection against ischemia–reperfusion injury[8] and transcriptional and protein alterations, consistent with the occurrence of a "second window of protection," were demonstrated in humans.[17]

How could emulsification of halogenated ethers enhance their cytoprotective actions in the heart (or other vital organs) and at the same time separate protection signaling from anesthetic effects? We speculate that large interfaces between ether-loaded micelles and lipid rafts may serve as ether-releasing reservoirs and form a cellular microenvironment promoting protection signaling. Volatile anesthetics partition in plasma membranes in accordance with the Meyer-Overton rule. Because of their physicochemical

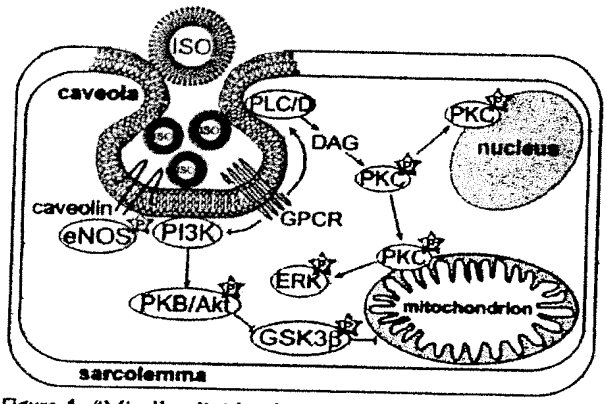

Figure 1. "Micelles–lipid raft" model. Isoflurane-loaded micelles closely interact with lipid rafts (not drawn to scale), including the subfamily of lipid rafts containing caveolin (named caveolae), reassembling key signaling molecules to signaling modules, which translate the signal to subcellular targets such as nuclei (transcriptional changes → "second window of protection") and mitochondria. P indicates phosphorylation of target proteins. In this proposed model, a large "sponge-like" water/lipid interface may be of particular importance (see text). ISO = isoflurane (or any other halogenated ether); PLC/D = phospholipase-C/D; DAG = diacyl glycerol; PKC = protein kinase C isoforms; GPCR = G-protein coupled receptors; eNOS = endothelial nitric oxide synthase; PI3K = phosphatidylinositol-3-kinase; PKB/Akt = protein kinase B; GSK3β = glycogen synthase-3β; ERK = extracellular signal-regulated kinase.

nature, ethers interact with polar and nonpolar molecules and thus favor amphiphilic binding cavities, typically occurring in the phospholipid bilayer of biological membranes.[18] Second, volatile anesthetics are not evenly distributed over the membrane, but are rather preferentially sequestrated in lipid rafts,[19,20] such as caveolae (a subclass of lipid rafts), which represent flask-shaped membrane invaginations at the cell surface enriched in cholesterol and sphingolipids (Fig. 1). Caveolae found on almost every cell type including cardiomyocytes (caveolin-3) and endothelial cells (caveolin-1), are small (10–300 nm), but may comprise as much as 25% of the total cell surface area. Since caveolae form specialized membrane units responsible for compartmentalization and organization of signaling molecules,[21] changes in protein–protein or protein–lipid interactions, as induced by halogenated ethers, potentially remodel the caveolar scaffold and affect cellular signaling. For example, the scaffolding protein caveolin-1 binds to endothelial nitric oxide synthase, a key player in myocardial protection, and thereby negatively regulates its activity.[22] Src protein tyrosine-kinase, a known isoflurane target,[23] phosphorylates caveolin promoting its dissociation, and thus activates endothelial nitric oxide synthase. G-protein coupled receptors such as adenosine receptors, important mediators of heart protection in pre- and postconditioning and capable of priming cardiac mitochondrial KATP channels,[24] were recently coimmunoprecipitated with caveolin indicating clustering

of these pivotal protective receptors in caveolae.[25] Further, caveolae are the sites where sarcolemmal phosphatidylinositol-4,5-bisphosphate is hydrolyzed by phospholipase C to generate diacylglycerol,[26,27] an activator of protein kinase C isoforms translating the preconditioning signal to subcellular targets.[28] Caveolae are also involved in the regulation of the phosphorylation status of protein kinase B (Akt) and extracellular signal-regulated kinases,[29] two important mediators of the (reperfusion injury salvage kinase) RISK pathway.[30] Halogenated ethers dissolved in the lipid phase of micelles may tightly interact with the "sponge-like" surface of lipid rafts over the large interfacial area, 1 mL of emulsion easily spans the area of 100 m^2, which is more than the surface of a human lung, and thus may activate protection signaling. Since lipid droplets exhibit high entrapment efficiency with sustained release, emulsified ethers may induce similar protection with lower total amounts of administered drug and in the absence of anesthesia. This would indeed be consistent with lower MAC values usually reported for emulsified versus inhaled ethers,[6] and further raise the possibility that protection observed with emulsified ethers might rather represent a combination of pre- and postconditioning due to the retention of "gas"-loaded lipid droplets in the tissue. In this proposed "micelles–lipid raft" model, the large organic/aqueous interface might be of particular interest.

First, the interfacial area improves stability and activity of phospholipases at cell membranes, enhancing formation of second messengers.[31,32] Second, interfaces accumulate lipid fragments such as acrolein,[33] which, in concert with ethers, might enhance activation of protein kinase C isoforms.[28,34] Third, water–lipid interfaces can separate enantiomers (nonsuperimposable mirror images of a drug),[32,35] which may exert differential cardioprotective effects, as previously shown for the racemic ketamine.[36,37] Interestingly, (+)-isoflurane exhibits stronger anesthetic effects than its (−)-stereoisomer, which, in contrast, provides superior ATP-preservation (2.5-fold) in anoxic cells.[38] Hence, one could speculate that differential partitioning of ether enantiomers at interfaces might help to separate cytoprotective from anesthetic effects, at least in the case of the racemic ethers isoflurane, enflurane, and desflurane. Taken together, in many aspects, the introduction of a carrier solvent for IV application of halogenated ethers exemplifies how closely related anesthetic mechanisms (with alterations in lipid bilayers and modulation of membrane-bound protein function) and protection signaling by preconditioning might indeed be.[39]

Determination of the ideal emulsion, particularly with respect to droplet size, surfactant properties, and stability, will be a crucial issue because emulsions are subject to various instability processes such as aggregation, flocculation, coalescence, and eventual phase separation, according to the second law of thermodynamics. Equally important, the effects of such emulsions on glucose-fatty acid metabolism and the reticuloendothelial system should be further elucidated. The energy substrate switch from fatty acid to glucose utilization is an important element in cardiac protection by halogenated ethers[40] and might be jeopardized by the coadministration of high concentrations of lipid-containing emulsions. These studies would first help to use the optimized formulation in in vivo human model systems of cell protection and preconditioning signaling,[8,17] and ultimately allow testing their utility in clinical trials of organ protection. Excitingly, emulsification of halogenated ethers not only allows IV and intrathecal, but also subcutaneous and intraperitoneal administration, of which each injection site shows a different (time course- and organ-wise) biodistribution. Finally, the use of an external magnetic field in combination with iron oxide-precoated micelles would allow efficient organ-targeted protection.

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